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Synthetic urokinase inhibitors as potential antitumor drugs

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IDrugs 2003 6(2):138-146
 ISSN 1369-7056

Urokinase-mediated plasminogen activation is involved in many normal physiological processes, including tissue remodeling, embryogenesis, wound healing and clot lysis. In addition, elevated levels of urokinase, the urokinase receptor uPA-R and its endogenous inhibitor plasminogen activator inhibitor (PAI-1), in combination with plasmin, play an important role in the pathogenesis of malignancy through its ability to mediate tumor cell growth, invasion and metastatic dissemination. The inhibition of urokinase with synthetic inhibitors is a new concept for a specific cancer therapy. This review examines synthetic urokinase inhibitors described during the last two years.

Keywords Tumor, urokinase

Introduction

Several proteases, like matrix metalloproteases (MMPs), the cysteine proteases cathepsin B and L, the aspartyl protease cathepsin D, and the serine proteases urokinase (uPA), plasmin and matriptase, are involved at multiple stages during the growth, invasion and progression of human tumors. High levels of expression of these proteases often correlate with poor prognosis for several cancer types. Consequently, there is hope that in addition to conventional forms of non-specific radiotherapy, chemotherapy and hormone therapy, new anticancer drugs can be developed by targeting one of these proteases using small molecule, synthetic inhibitors. During the last decade a major effort in the pharmaceutical industry was concentrated on the inhibition of MMPs. However, some disappointing results regarding poor therapeutic benefit and side effects observed during clinical studies stimulated the search for other inhibitor types. From experimental work using first generation inhibitors, uPA emerged as an attractive target for drugs designed to reduce tumor invasion [1••,2,3].

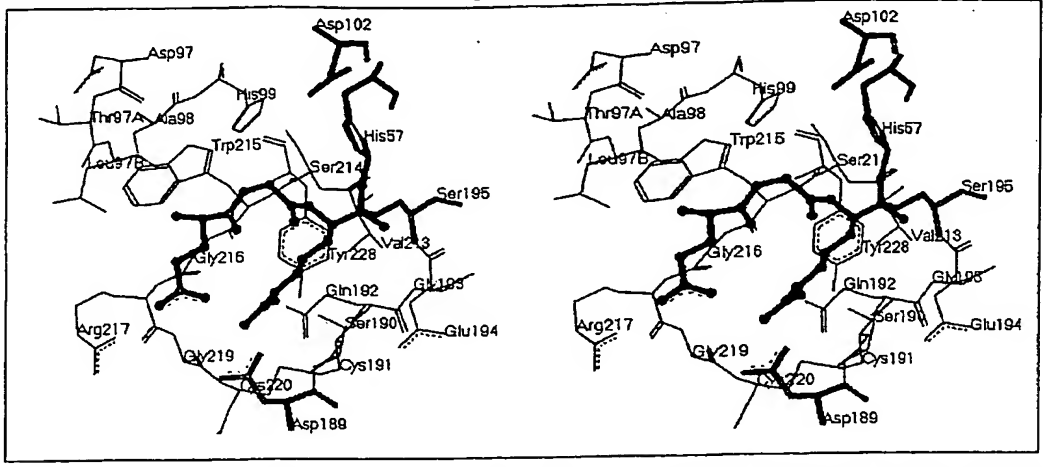
uPA and the second plasminogen activator tPA belong to the trypsin-like serine protease family and activate plasminogen into the serine protease plasmin after cleavage of its Arg^{561}-Val^{562} peptide bond. Enzymatically active uPA and tPA variants have a long history as thrombolytic agents, but significant differences exist between these enzymes. Due to its high affinity for fibrin and activation by fibrin binding, the main biological role for tPA seems to be associated with fibrinolysis. tPA does not bind to tumor cell surface receptors or promote tumor cell-focused proteolysis. In contrast, uPA is a central molecule in pericellular proteolysis, produced by a variety of normal and tumor cells as an almost inactive single-chain pro-enzyme (pro-uPA). These cells also express a specific surface receptor (uPA-R) for pro-uPA. After binding to its receptor, pro-uPA is converted into enzymatically active uPA by plasmin more rapidly than in its unbound state when free in the fluid-phase. This cell surface-focused uPA generates additional plasmin with an increased activity, which can activate several pro-forms of MMPs and, in turn, more uPA. These proteases promote tumor cell invasion through local proteolysis of the surrounding extracellular matrix proteins [4•,5,6]. As well as its proteolytic activity, uPA can induce other biological processes, which encompass mitogenic, chemotactic, adhesive and migratory properties important for tumor growth, motility and angiogenesis [7]. Some of these processes are induced by intracellular signal transduction events after uPA's ligation with its receptor [8,9]. uPA activity is controlled by the serpins plasminogen activator inhibitor (PAI)-1 and PAI-2. The ratio of PAI-1 to PAI-2 can be used as a diagnostic marker; high levels of PAI-1 indicate a poor prognosis for node-positive and node-negative breast cancer. The ternary complex of PAI-1-inhibited high molecular weight (HMW)-uPA bound to uPA-R is internalized and initiates signal transduction and cell proliferation. After internalization uPA-R is recycled to the cell surface and focuses the proteolytic system back to the invasive front of the cell [10]. In contrast, the PAI-2 and receptor-bound uPA complex is not internalized; intravenous injection of a PAI-2-expressing adenovirus led to efficient lung metastasis reduction [11].

There are several potential ways of modulating the activity of uPA. One strategy is to block uPA ligation with its receptor with antibodies or competitive analogs. Another method is to interfere with the expression of uPA, uPA-R or PAI-1 at the gene or protein level. A third possibility would be the inhibition of intracellular signal transduction events. An additional strategy is the direct inhibition of uPA by small molecule active site inhibitors, which is described in this report.

Structure of uPA

Pro-uPA consists of 411 amino acids and forms three distinct protein domains. The binding site for uPA-R is located in an N-terminal growth-factor-like domain, which is followed by a kringle domain and the serine protease domain with the catalytic center. Activation of pro-uPA (cleavage within the serine protease domain by different proteases) leads to enzymatically active HMW-uPA [12]. The first X-ray crystal structure of the activated serine protease domain from urokinase in complex with the irreversible inhibitor H-Glu-Gly-Arg-chloromethyl-ketone demonstrated that it has the typical topology found for other trypsin-like serine proteases (Figure 1) [13••]. However, there are some differences between the active site region of uPA and other members of this protease family that are important for inhibitor design. Adjacent to Asp^{189} at the bottom of the S1 pocket, uPA contains a serine in position 190, found also in trypsin, plasmin and Factor VIIa. This residue is useful for designing selective molecules compared to proteases of the Ala^{190}-subclass, which are also important therapeutic targets (thrombin, Factor Xa, plasma-kallikrein and tPA). In position 99, human uPA has a unique histidine that limits the space of the S2 site, therefore, it preferentially accepts small P2-residues (Gly, Ala) in substrates and inhibitors. The S3/S4 pocket is reduced in size due to the 97-insertion loop

Figure 1. Stereo view of selected residues in the active site region of uPA in complex with H-Glu-Gly-Arg-chloromethylketone.



The inhibitor is drawn using black balls and sticks. The amino acids of the catalytic triad (Ser^{195}, His^{57}, Asp^{102}) and Asp^{189} at the bottom of the S1-pocket are shown as gray sticks: all other uPA-residues appear as black lines. The uPA-residues His^{57} and Ser^{195} form two covalent bonds with the arginyl-ketone moiety of the inhibitor. Several additional hydrogen bonds are formed between the inhibitor and uPA (eg, the guanidino group of P1-Arg and Asp^{189}, the NH group of P1-Arg and the carbonyl oxygen of Ser^{214}, the NH group of P2-Gly and the side chain of His^{99}, the amino group of P3-Glu and the carbonyl oxygen of Leu^{97B}, and the carboxyl side chain of P3-Glu and the guanidino group of Arg^{217} in uPA). The 3-dimensional coordinates for this figure were taken from the 1lmw.pdb file on the Protein Data Bank website [48].

consisting of Thr^{97A} and Leu^{97B}; D-serine is a preferred P3 amino acid in substrate analog uPA-inhibitor structures. Recently, additional crystal structures of the uPA B-chain bound to synthetic inhibitors have been described. In all cases uPA mutants that lose their A-chain after activation of the serine protease domain were used for crystallization [14,15,16•].

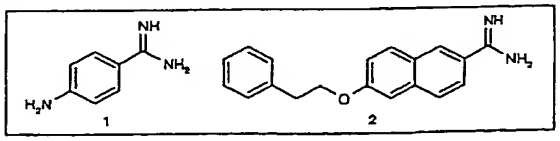
Development of uPA inhibitors

Compared with other trypsin-like serine proteases, especially thrombin or Factor Xa, few basic structures are known which selectively block uPA. Most of the potent uPA inhibitors described so far are non-peptidic structures that contain an amidino- or guanidino-substituted aromatic system as the P1 residue. Tripeptide-derived inhibitors with improved selectivity have also been developed.

Non-peptidic benzamidine and naphthamidine derivatives

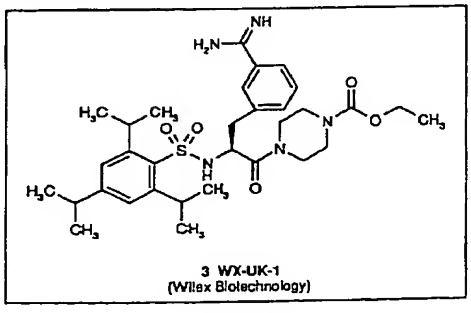
Substituted benzamidines, such as compound 1, and 2-naphthamidines, such as compound 2 (both Figure 2), identified by Stürzebecher and colleagues, demonstrated a moderate uPA affinity with micromolar inhibition constants [17•]. Following this, the benzamidine moiety was transferred into non-proteinogenic amino acids (3- and 4-amidinophenylalanines and their homologs) to allow for simple N- and C-terminal elongation of the inhibitors. However, most of these compounds demonstrated poor inhibition of uPA.

Figure 2. Non-peptidic benzamidine and napthamidine derivatives.



Pentapharm AG and the Medical School of Erfurt identified inhibitors, containing an N-terminal 2,4,6-triisopropyl-phenylsulfonyl residue and a substituted piperazide at the C-terminus [18,101]. The most potent inhibitor, WX-UK-1 (3, Wilex Biotechnology GmbH; Figure 3), with a K_i value of 0.41 µM, demonstrates remarkable potency in inhibiting tumor growth and metastasis [102]. WX-UK-1 is in clinical development as part of a combination treatment with chemotherapy for patients with various cancer types (breast, ovarian or gastric) who have elevated levels of uPA in their tumors. In March 2002, Wilex announced the completion of a phase Ia clinical study in 18 healthy volunteers. The drug was safe and well tolerated at all doses tested, with no reported serious adverse events [19].

Figure 3. The structure of WX-UK-1.



3 WX-UK-1
(Wilex Biotechnology)

On the basis of crystallographic data, 2-naphthamidine (K_i = 5.9 µM) was chosen as the lead scaffold for structure-directed optimization by Abbott Laboratories. In a first series, only position 8 was substituted. The 8-aminopyrimidine and 8-methylcarbamyl groups in the most potent compounds, 4 and 5 (both Figure 4; see Table 1), occupy a shallow

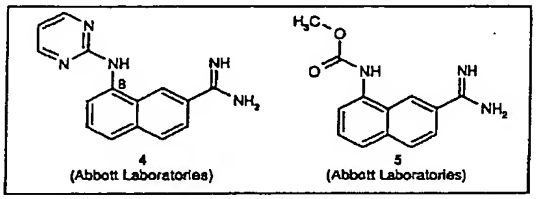
Table 1. Inhibition of uPA and related enzymes.

Compound	K_i or IC_{50} values (μM)					Reference
	uPA	tPA	Plasmin	Trypsin	Thrombin	
4	0.03	23	3.8	1.6	3.9	[20]
5	0.04	1.8	40	0.3	5.2	[20]
6	0.00064	ni	ni	ni	ni	[21•]
7	0.00092	ni	ni	ni	ni	[21•]
8	0.01	ni	ni	ni	ni	[104]
9	0.01	ni	ni	ni	ni	[104]
11	0.32*	107*	352*	4.9*	850*	[1••]
12	0.07*	24*	> 250*	2.8*	> 250*	[1••]
13	0.25	3.4	1.7	1.4	2.3	[25]
14	0.008	0.035	0.1	0.13	0.32	[26•]
15	0.009	8.8	0.11	0.23	60	[26•]
19	0.06	ni	ni	ni	ni	[28]
20	0.044	ni	ni	ni	ni	[29]
21	0.101	ni	ni	ni	ni	[30]
22	7.0	> 1000	> 1000	32	> 1000	[31•]
23	6.1	> 1000	> 1000	120	> 1000	[32•]
24	2.4	> 1000	> 1000	46	600	[33]
25	2.9	ni	ni	ni	ni	[34]
26	0.49	ni	ni	ni	ni	[35]
27	0.17	ni	ni	ni	ni	[35]
30	0.0031*	> 2.5*	0.367*	ni	ni	[36•]
32	0.023*	> 2.5*	1.46*	ni	ni	[36•]
33	0.0077	ni	0.54	0.0033	0.11	[37•]
34	0.036	ni	11	0.15	13	[37•]

*IC_{50} values; ni, not indicated in the literature.

subpocket termed S1β, which is formed by the uPA-residues Gly^{218}, Ser^{146}, the Cys^{191}-Cys^{220} disulfide bridge, the side chain of Lys^{143} and part of Gln^{192} [20,103].

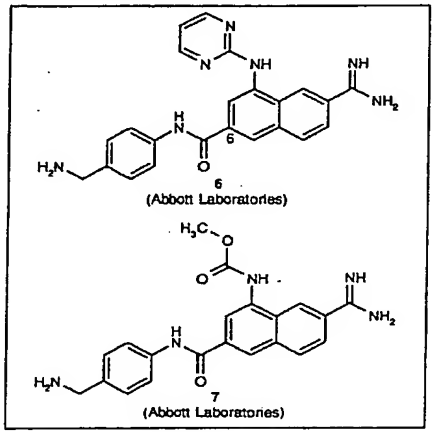
Figure 4. 8-Substituted 2-napthamidine derivatives by Abbott.



The activity could be further improved by addition of an aromatic residue at position 6 of the naphthamidine containing a basic aminomethyl group, eg, compounds 6 and 7 (both Figure 5). The phenyl ring makes an aryl-aryl interaction with His^{57} of the catalytic triad and the aminomethyl group interacts with the carboxylate side chain of Asp^{60} found in human uPA [21•]. These analogs are significantly less active toward mouse uPA, which contains a glutamine in position 60. The K_i values for uPA correlate well with the activity of these inhibitors in a cell-based assay, which measures the plasmin-catalyzed fibronectin degradation after cell-surface urokinase-mediated activation of plasminogen.

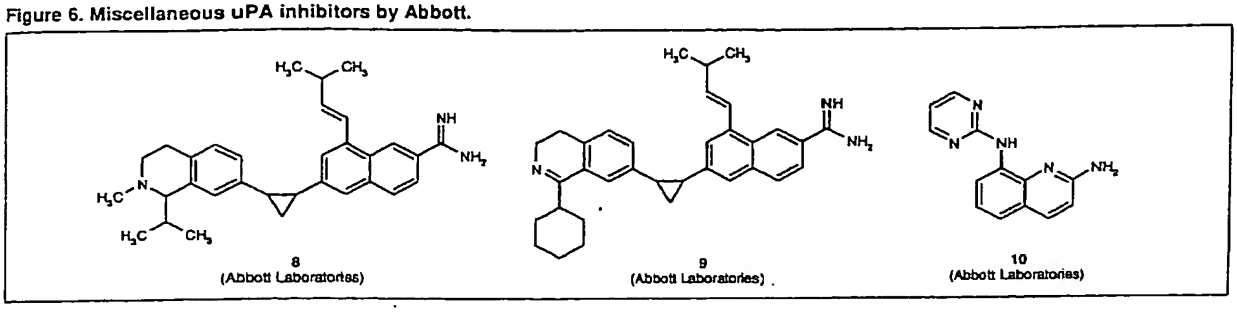
In an additional patent, Abbott claimed analogs, such as compounds 8 and 9 (both Figure 6), with K_i values of 10 nM,

Figure 5. 8- And 6-substituted 2-napthamidine derivatives by Abbott.



containing a cyclopropyl ring instead of the peptide bond between both aromatic systems [104]. In general, there is only limited information about the pharmacokinetic properties of this inhibitor type, although compound 4, a 2-naphthamidine, is not orally absorbed. An X-ray crystallography-driven screening technique followed by chemical lead optimization was used to identify the structurally related but less basic 8-aminopyrimidyl-2-aminoquinoline 10 (K_i = 0.37 μM; Figure 6), which is 38% orally bioavailable [22].

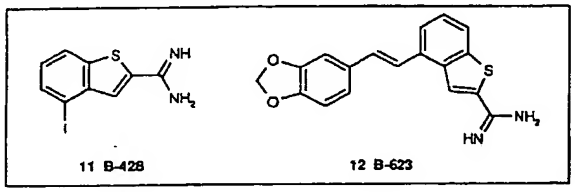
Figure 6. Miscellaneous uPA inhibitors by Abbott.



Amidino-substituted heterocycles

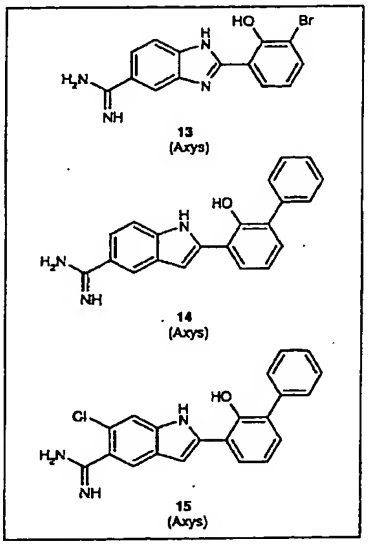
In 1993, Towle *et al* described the benzo[*b*]thiophene-2-carboxamidines B-428 (11; Figure 7) and B-623 (12; Figure 7), which remained the most potent urokinase inhibitors for many years, but by December 1999, Eisai Co Ltd had discontinued their development. The same research group demonstrated for the first time that these analogs are able to inhibit not only free but also cell surface-bound uPA, as well as cell surface uPA-mediated cellular degradative functions, suggesting that this class of compounds may hold significant promise as anti-invasiveness drugs [1••,23].

Figure 7. The structures of B-428 and B-623.



Axys Pharmacueticals Inc claimed several 5-amidino-benzimidazoles, eg, compound 13 (Figure 8), and 5-amidino-indoles, eg, compound 14 (Figure 8), substituted with a 2-phenol moiety [105]. The phenolic hydroxyl group and the NH of the benzimidazol or indol are involved in forming a cluster of very short hydrogen bonds to the uPA-residues Ser^{195}, His^{57} and Gly^{193}, and two water molecules, which is important for high inhibitory potency [24]. Changing the benzimidazole heterocycle to an indole, thereby fixing the tautomeric nature of the nitrogen compared to the benzimidazole analogs, resulted in a 2- to 8-fold potency enhancement. The uPA-affinity was further improved by incorporation of an additional phenyl group which is directed toward S1' and makes van der Waals contact with the disulfide bridge between Cys^{42} and Cys^{58}, and His^{57} and Val^{41} [25]. The specificity of this relatively non-selective scaffold could be amplified for uPA and all other trypsin-like proteases which contain a serine at position 190 (plasmin, trypsin, Factor VIIa) against the Ala^{190} enzymes (tPA, plasma-kallikrein, thrombin, Factor Xa) by incorporation of a halo group *ortho-* to the amidine [26•,27]. This halo group, eg, in compound 15 (Figure 8), displaces an important water molecule in the S1 subsite and eliminates a key hydrogen bond. In the Ser^{190} enzymes the affinity is maintained since the hydroxyl oxygen of Ser^{190} compensates for the displaced water molecule.

Figure 8. 5-Amidino-benzimidazole and -indole derivatives by Axys Pharmaceuticals.



In a recent patent application an additional tetrazol ring was incorporated, eg, compound 16 (Figure 9), however, no information about the potency of these compounds was given [106]. In addition, Axys published a series of acylated 4-aminobenzamidines, eg, compounds 17 and 18 (both Figure 9); a halo atom *ortho-* to the P1 amidine was incorporated, however, fluorine only slightly improved selectivity whereas the chloro derivative was much less potent [27].

Figure 9. 5- Amidino-indole and 4- aminobenzamidine derivatives by Axys.

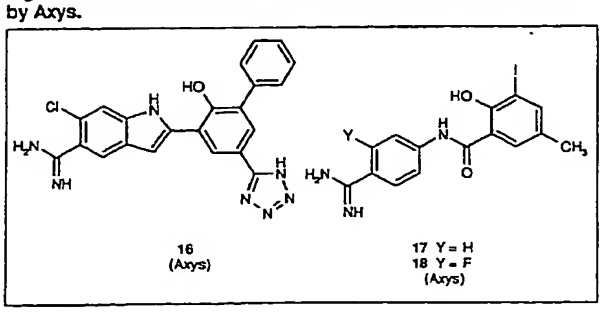
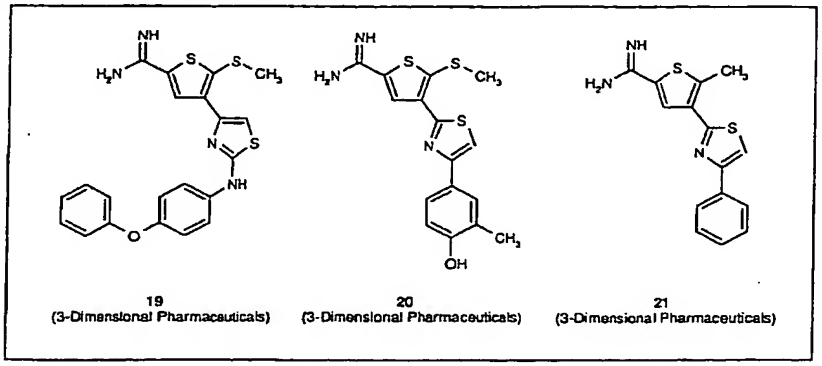


Figure 10. uPA Inhibitors by 3-Dimensional Pharmaceuticals.



During screening of an amidine library, 3-Dimensional Pharmaceuticals Inc identified 2-amidino-5-thiomethyl-thiophene as an active uPA inhibitor (K_i = 6 µM) [107,108]. In a first series, the thiophene was substituted with 2-aminothiazole groups, which resulted in several compounds with K_i values of < 100 nM, eg, compound 19 (K_i = 60 nM; Figure 10) [28]. In a second report, the 2-aminothiazole was replaced by an aryl-substituted thiazole; the most potent compounds had K_i values of ~ 50 nM, such as compound 20 (K_i = 44 nM; Figure 10) [29]. These analogs possess high uPA selectivity and are able to inhibit tumor metastasis in a cell-based assay. However, a drawback of these compounds is their marginal solubility. Therefore, the 5-methylthio group was replaced by a simple methyl group, eg, compound 21 (K_i = 103 nM; Figure 10), which maintained the potency and significantly improved the solubility of these inhibitors [30].

Guanidino-substituted aromates

One of the first compounds within the guanidine-substituted aromate series was the diuretic drug amiloride (22; Figure 11), which inhibits uPA with a K_i value of 7 µM [31•] and served as a prototype uPA inhibitor for X-ray crystallography [14,16•]. Yang *et al* disclosed substituted phenylguanidines, with the most potent and selective derivative, compound 23 (K_i = 6.1 µM; Figure 11), containing a chlorine atom at the 4-position [32•]. This lead was improved by elongation with hydrophobic substituents at position 4 of the phenyl ring. The analog with the highest uPA affinity is the urea derivative WX-293 (24, Wilex Biotechnology GmbH; Figure 11), which had a K_i value of 2.4 µM [109]. The hydrophobic adamantyl group of WX-293 is directed toward the Cys^{58}-Cys^{42} disulfide bridge into a shallow S1' subsite and does not occupy the non-primed region of uPA [33,16•]. As expected, the guanidinophenyl moiety binds to the S1 pocket and the ureido group is involved in four defined (partially water-mediated) hydrogen bonds.

2-Pyridinylguanidines have recently been published by Pfizer Inc. The affinity of the inhibitors from a first series, eg, compound 25 (K_i = 2.9 µM; Figure 12) [34], was improved by incorporation of a rigid aryl-containing side chain at position 3 of the pyridine, eg, compounds 26 and 27 (K_i values of 0.49 and 0.17 µM, respectively; Figure 12) [35]. In addition, Pfizer disclosed closely related compounds (eg, 28 and 29; both Figure 12) based on a 1-guanidino-4-chloro-isoquinoline template with K_i values of < 20 nM [110,111]; however, the selectivity of the compounds was not indicated.

Figure 11. Guanidino-substitued aromates.

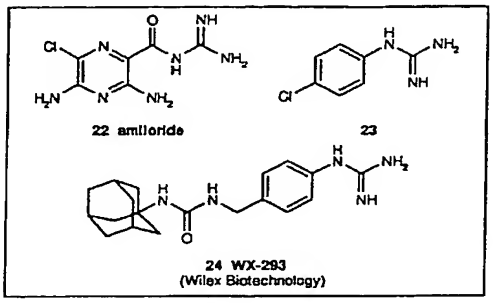
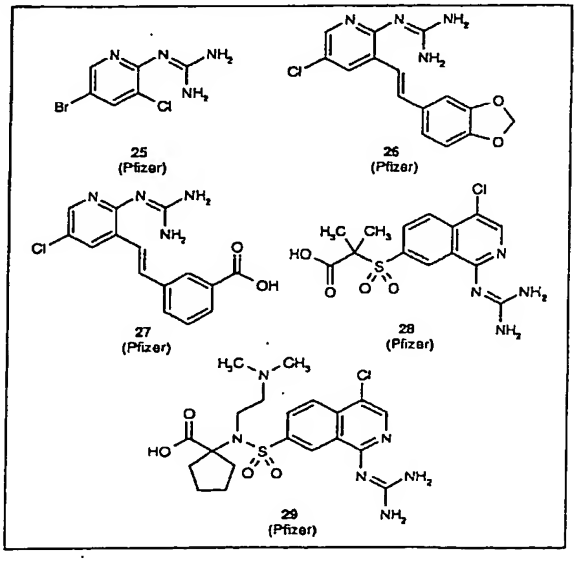


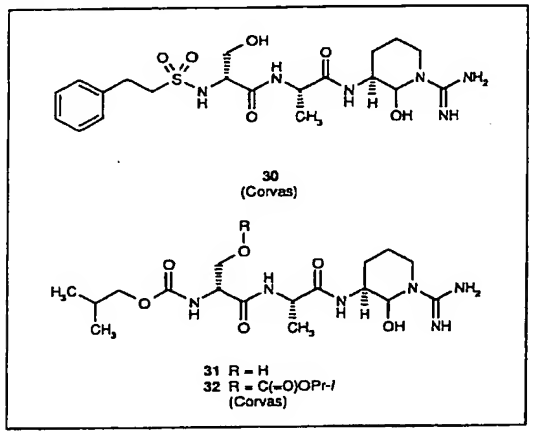
Figure 12. Guanidino-substitued aromates by Pfizer.



Peptide derivatives

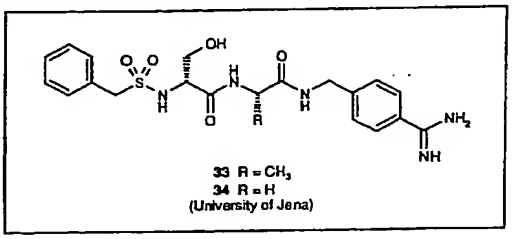
Using a library approach, Corvas International Inc identified tetrapeptide inhibitors with an arginine-mimic aldehyde or an arginine ketoamide group at the P1 position, and a D-serine at P3 [112]. Compound 30 (Figure 13) showed the highest potency within this series, with an IC_{50} value of 3.1 nM [36•]. In order to improve the half-life of these compounds, Corvas applied a prodrug strategy using a carbonate-type protection on the P3-D-Ser side chain. Compound 31 (Figure 13) is easily converted from compound 32 (Figure 13) in rats after subcutaneous administration. The apparent terminal elimination half-life of compound 31 (applied as compound 32) is 10.7 h, with a relative bioavailability of ~ 87%.

Figure 13. Peptide derivatives by Corvas I.



A group from the University of Jena (Germany) used this motif with a D-serine in the P3-position and replaced the P1-arginal with a 4-amidinobenzylamide [113], which is a decarboxylated arginine mimetic that arose from the development of AstraZeneca plc's thrombin inhibitor melagatran. In contrast to the arginal series, these analogs are classical, fast-binding inhibitors without the potentially labile stereogenic center present in the arginine-based transition-state analogs. The highest uPA potency within this series was found for the P2-Ala derivative compound 33 (K_i = 7.7 nM; Figure 14), whereas a more pronounced selectivity was observed for the Gly-inhibitor compound 34 (K_i = 36 nM; Figure 14) [37•]. Within this series, it was also demonstrated that P3-carbonate and P1-hydroxyamidine prodrugs are transformed to compound 34 after subcutaneous injection in rats.

Figure 14. Peptide derivatives by University of Jena.

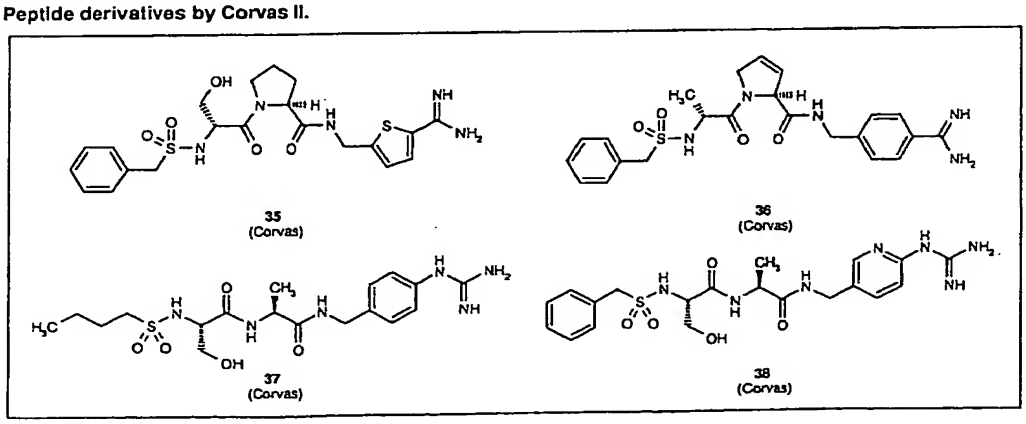


In a recent patent application [114], Corvas expanded their arginal inhibitors by incorporation of different P1 mimetics, such as 4-amidinobenzylamine, 2-amidinothiophen-5-methylamine, 2-guanidinothiophen-5-methylamine, 4-guanidinobenzylamine, 3-guanidinobenzylamine, 2-guanidinopyridyl-5-methylamine, 3-amidinopyridyl-5-methylamine and agmatine. The 66 examples given in the patent show that these residues can be combined with a variety of P4-sulfonyl groups and P2 amino acids. Compounds 35 to 38 (all Figure 15) have K_i values of < 100 nM (exact values are not indicated in the patent). Surprisingly, compound 36 with a D-Ala in the P3-position also demonstrated comparable uPA affinity.

Synthetic uPA-inhibitors in animal studies

Considerable experimental evidence supports the importance of the uPA/uPA-R system in plasmin and MMP-activation, in promoting tumor cell proliferation, invasion, metastasis and angiogenesis. However, to date, there exist only a few reports that clearly demonstrate the efficacy of low molecular weight uPA inhibitors as antitumor drugs in animal experiments; no

Figure 15. Peptide derivatives by Corvas II.



phase II or III clinical studies have been performed thus far. This may be due to a lack of highly specific and effective synthetic uPA inhibitors, as only in recent years have more potent leads (K_i < 50 nM) been described. Therefore, most of the animal studies have been performed using amiloride and the benzothiophenes B-428 and B-623, which have only moderate uPA affinity.

Amiloride, administered in drinking water, reduced the number of pulmonary metastases in rats, which were inoculated with mammary cancer cells [38]. In addition, there exist reports that amiloride at a dose of 7.5 mg/kg/day can suppress colon carcinogenesis [39], and at a dose of 5.0 mg/kg/day leads to a reduced incidence of tumor metastases in the peritoneum of rats [40]. However, amiloride was ineffective in inhibiting tumor growth and metastasis in rats bearing tumors from a highly aggressive prostate cancer cell line [41].

In contrast to amiloride, B-428 was able to reduce tumor growth and invasiveness in a rat prostate cancer model [42]. Daily treatment with B-428 (20 mg/kg/day ip) and B-623 (7.5 mg/kg/day ip) for 2 weeks, beginning after tumor-take, markedly blocked the invasion of the muscle and adipose layers of the subcutis and dermis in mice bearing highly invasive subcutaneous F3II tumors, established from a mammary adenocarcinoma cell line [2]. However, in the same *in vivo* model, B-428 and B-623 did not demonstrate antimetastatic effects. A series of animal studies have also been conducted using WX-UK-1, which was highly effective in preventing tumor growth and metastasis formation in rat pancreatic and mammary tumor models [102].

Due to the poor efficacy and conclusions drawn from the clinical studies with MMP inhibitors as anticancer drugs [43••], some points have to be considered for future work with uPA inhibitors. MMP and uPA inhibitors block the same proteolytic cascade, which is important for the degradation of extracellular matrix proteins. However, it must still be demonstrated that uPA is a more effective target because it is located at the beginning of this pathway. One advantage of blocking uPA activity compared to MMP inhibition may be the effect on angiogenesis, which is important for tumor progression. Because MMPs have been implicated in the generation of molecules with anti-angiogenic activity (eg, angiostatin), MMP inhibition may result in the stimulation rather than the inhibition of angiogenesis [44]. In contrast, in a chicken embryo chorioallantoic membrane model, all uPA inhibitors tested (amiloride, benzamidine, B-428 and B-623) caused a significant reduction in angiogenesis [45•].

Another point is the development of more appropriate animal models, which more closely mimic human cancers. Models of subcutaneous or intravenous injection of human tumor cells into immunodeficient mice may be inadequate to evaluate the efficacy of protease inhibitors because they do not mirror host-tumor interactions [43••]. An additional problem is that in animal models, protease inhibitor treatment normally starts with or even before inoculation of tumor cells and is maintained throughout tumor progression, whereas clinical experiments, in the case of MMP inhibitors, are usually performed with late-stage patients. In animal experiments, the MMP inhibitor batimastat reduced tumor burden in mice when administered at both early and intermediate stages of the disease, but had no effect on mice with advanced tumors [46]. Based on this experience, the effects of uPA inhibitors at different steps of tumor progression in animal models must be investigated.

Usually, only human uPA is used for enzyme kinetic experiments *in vitro*, however, with regard to the efficacy of synthetic uPA inhibitors, it is important to consider species-dependent differences in the uPA sequence, which could distort the conclusions derived from animal studies. Although the residues of the catalytic triade (57, 102 and 195) and the P1 specificity pocket (187-197, 212-229) are highly conserved among uPAs of different species, in some cases, differences exist between residues which are close to the active site and important for inhibitor binding (eg, human and mouse uPA differ in residues 60, 99, 146 and 192) [21•,47].

Conclusion

The increasing knowledge about the importance of the plasminogen activator system in carcinogenesis, especially in metastasis formation and invasion, has stimulated the search for more effective uPA inhibitors to block cell surface-associated proteolysis. In the meantime several X-ray structures of uPA/inhibitor complexes have been solved, which demonstrate similarities as well as significant differences between uPA and other members of the trypsin-like serine protease family. This information accelerated the development of more potent and specific uPA inhibitors, and several new lead compounds are now suited for further preclinical studies to evaluate their efficacy as antitumor drugs.

Most of the compounds are derived from benzamidine, naphthamidine or amidino- and guanidino-substituted heterocyclic compounds. Therefore, nearly all of the potent uPA inhibitors described so far still contain a strong basic P1-residue. Little information is available concerning whether these drugs have a significant oral bioavailability and suitable half-life. Although an oral application of such inhibitors would be advantageous, it is not an absolute requirement of antitumor drugs. A parenteral application could also be efficacious. Examples of prodrugs with improved pharmacokinetic properties have also been described.

Future work should evaluate newly developed uPA-inhibitors in appropriate animal models, where the attention should be directed towards the efficacy of these compounds dependent on the stage and type of tumor. This requires a careful analysis of the expression pattern of uPA in the individual situation to allow for a rational decision on whether uPA is an appropriate target enzyme or not. Almost nothing is known about the efficacy of a combination therapy using synthetic uPA inhibitors together with other cytostatic or cytotoxic agents.

Interestingly, in a recent patent application it was claimed that urokinase inhibitors can also be used for the treatment and prevention of pulmonary hypertension, cardiac remodeling and subsequent cardiac failure [115]. Further definitive studies in this field are awaited.

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